The American Journal of Agriculture and Biomedical Engineering
ISSN 2689-1018 Volume 08 - 2026

Reduction of Platelet Hyperactivity by Polyphenol in An Experimental Model of Alzheimer's Disease in Rats

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Received: 25 Jan 2026 | Received Revised Version: 15 Feb 2026 | Accepted: 28 Feb 2026 | Published: 17 Mar 2026

Volume 08 Issue 03 2026 | Crossref DOI: 10.37547/tajabe/Volume08Issue03-03

Abstract

Background: Alzheimer's disease (AD) is increasingly associated not only with neuronal degeneration but also with platelet dysfunction, altered hemostasis, and calcium-dependent peripheral signaling abnormalities. Objective: To evaluate the effects of G-40 polyphenol on platelet aggregation, coagulation hemostasis, and intracellular calcium mobilization in a rat AD-like condition. Methods: Platelet-rich plasma and platelet suspensions obtained from control rats and rats with an AlCl3-induced AD-like condition were analyzed. Platelet aggregation was assessed by Born aggregometry after stimulation with ADP, adrenaline, and collagen. Coagulation was evaluated using thrombin time (TT), activated partial thromboplastin time (APTT/QFTV), and prothrombin time (PT, TechPlastin). Intracellular calcium was monitored in Fluo-4 AM-loaded platelets after ADP stimulation, with EGTA used as a calcium-chelating control. G-40 was tested primarily at 50 uM, with concentration-response evaluation in the 10-100 uM range for calcium readouts. Results: The AD model displayed spontaneous platelet aggregation and enhanced agonist-induced aggregation relative to controls. G-40 partially inhibited ADP- and collagen-induced aggregation in both normal and AD conditions. The AD model shortened

TT, APTT, and PT, indicating a procoagulant shift, whereas G-40 markedly prolonged all three parameters. G-40 also suppressed ADP-induced intracellular Ca2+ mobilization by 40-55%, with 50 uM showing the greatest effect. Conclusions: G-40 exerts a multi-target antiplatelet and anticoagulant action in the AD-like state. Its effects are most consistently explained by attenuation of Ca2+-dependent platelet activation together with interference at common coagulation pathway steps. These findings support G-40 as a promising candidate for correcting platelet hyperreactivity and hemostatic imbalance associated with AD.

Keywords: Alzheimer's disease; platelets; aggregation; hemostasis; polyphenol; G-40; intracellular calcium; ADP.

Cite This Article: Yuldasheva G.K., Holiqova M.A., Muratova M.X., Kozokov I. B., Khoshimov N.N., Erkinov I. O., … Abduganiyeva M.A. (2026). Reduction of Platelet Hyperactivity by Polyphenol in An Experimental Model of Alzheimer's Disease in Rats. The American Journal of Agriculture and Biomedical Engineering, 8(03), 10–16. https://doi.org/10.37547/tajabe/Volume08Issue03-03

1. Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by cognitive decline, synaptic dysfunction, oxidative stress, neuroinflammation, and beta-amyloid (Abeta) accumulation [1-4]. In recent years, increasing attention has been paid to the vascular and hemostatic components of AD, including platelet activation, fibrin(ogen) abnormalities, and a prothrombotic shift in the peripheral circulation [1-5]. Platelets are particularly relevant because they store and release amyloid precursor protein (APP), interact with Abeta species, contribute to vascular amyloid pathology, and function as an accessible peripheral model for AD-related signaling disturbances [5,6,16-18].

Platelet activation is a calcium-dependent multistep process. Agonists such as ADP, adrenaline, and collagen converge on signaling cascades that increase cytosolic free calcium, trigger granule secretion, enhance thromboxane A2 formation, and activate integrin alphaIIbbeta3, thereby stabilizing aggregation [6,7]. In AD-like conditions, oxidative stress and Abeta-driven signaling may enhance platelet sensitivity, promote spontaneous aggregation, and amplify responses to physiological agonists [2-5,16-18]. Thus, platelet hyperreactivity may represent both a mechanistic readout and a functionally relevant peripheral manifestation of AD pathology [19-24].

Polyphenols are attractive candidates for correcting these alterations because many compounds in this class combine antioxidant, membrane-stabilizing, antiplatelet, and anticoagulant properties [8-10,15,25-27]. However, the effects of G-40 polyphenol on platelet hyperreactivity, plasma coagulation kinetics, and intracellular Ca2+ transport under AD-like conditions have not been systematically described [28,29]. Therefore, the aim of this study was to analyze platelet aggregation, coagulation hemostasis, and platelet intracellular Ca2+ mobilization in an experimental AD-like rat model and to determine how G-40 modulates these changes.

2. Methods

This study was based on biomaterials obtained from male albino rats used in the dissertation protocol. Animals with an experimental AD-like condition were generated by aluminum chloride (AlCl3) exposure according to the model-development procedure described in the dissertation, while matched untreated animals served as controls. Blood was collected into 3.8% sodium citrate (9:1, blood:anticoagulant). Platelet-rich plasma (PRP) and platelet-poor plasma were obtained by sequential centrifugation of citrated whole blood according to the protocol used in the dissertation.

Platelet aggregation was recorded by the Born optical aggregometry method [11] using an ALAT-2 Biola aggregometer. PRP was stimulated with ADP, adrenaline, or collagen in the low-micromolar range described in the dissertation protocol. For inhibitory assays, G-40 polyphenol was added before agonist stimulation; the main analytical concentration was 50 uM. Spontaneous aggregation was evaluated in unstimulated PRP. Aggregation traces were analyzed as irreversible

aggregation responses and expressed as percentage change relative to the optical baseline.

Coagulation hemostasis was assessed in plasma using thrombin time (TT), activated partial thromboplastin time (APTT), and prothrombin time (PT) with a TechPlastin-based assay. These tests were selected to interrogate the final fibrinogen-to-fibrin step, the intrinsic/common pathway, and the extrinsic/common pathway, respectively. G-40 was evaluated under control and AD-like plasma conditions to determine whether its action was restricted to a single branch of the cascade or extended to common downstream coagulation steps.

To study platelet intracellular calcium, platelets were loaded with Fluo-4 AM. According to the dissertation protocol, a 10 mM Fluo-4 AM stock solution was prepared in DMSO and applied to platelet suspensions in Krebs-Ringer buffer, followed by incubation for 30 min at 37 C. ADP (5 uM) was used to stimulate intracellular Ca2+ release. In selected experiments, EGTA was included as a calcium-chelating control. G-40 polyphenol was added 2 min before ADP stimulation in concentration-response experiments (10-100 uM) and in the principal 50 uM condition.

Data are presented as mean or mean +- SEM. Normality was assessed with the Shapiro-Wilk test. Group comparisons were performed according to the design using two-way ANOVA (factors: model x treatment) followed by appropriate post hoc tests. For in vitro concentration-response experiments (0-100 uM), one-way ANOVA with Dunnett correction versus control was recommended and applied where appropriate. Statistical analysis and graph preparation were performed in OriginPro 2022 (OriginLab, USA). A value of $p < 0.05$ was considered statistically significant.

3. Results

Platelet function in the AD-like model shifted toward a hyperreactive phenotype. In control PRP, spontaneous aggregation was not observed, whereas spontaneous aggregation appeared in PRP from AD-model rats. ADP produced an irreversible biphasic aggregation pattern of 60-90% in control samples, and this response increased by 10-15% under AD-like conditions. Adrenaline induced 45-70% irreversible aggregation in controls and was 15-20% higher in the AD model. Collagen evoked 50-80% irreversible aggregation in controls and increased by 12-18% in the AD condition. These findings indicate generalized platelet hypersensitivity rather than an isolated defect in a single agonist pathway (table-1).

Table 1. Platelet aggregation profile and inhibitory effects of G-40 polyphenol

Inducer / condition	Control	AD-like model	G-40 (50 uM) in control PRP	G-40 (50 uM) in AD PRP
Spontaneous aggregation	Not observed	Observed	-	-
ADP (5-10 uM)	60-90% irreversible aggregation	10-15% above control	23% inhibition	20% inhibition
Adrenaline (5-10 uM)	45-70% irreversible aggregation	15-20% above control	Not quantified	Not quantified
Collagen (5-10 uM)	50-80% irreversible aggregation	12-18% above control	22% inhibition	18% inhibition

PRP, platelet-rich plasma.

G-40 polyphenol partially suppressed platelet aggregation in both normal and AD-like conditions. In control PRP, G-40 at 50 uM inhibited ADP-induced aggregation by 23% and collagen-induced aggregation by 22%. In PRP obtained from AD-model rats, G-40 at the same concentration inhibited ADP-induced aggregation by 20% and collagen-induced aggregation

by 18%. Although inhibitory efficacy was slightly lower in the AD background, the effect remained clearly detectable, indicating that G-40 retains biological activity even when platelets are primed toward hyperreactivity.

Coagulation testing showed that the AD-like model also shifted plasma hemostasis toward a procoagulant state. TT decreased from 25 s in controls to 11 s in AD plasma. APTT decreased from 30 s to 20 s, and PT decreased from 26 s to 20 s. In contrast, G-40 at 50 uM markedly prolonged all three coagulation readouts: TT to 47 s, APTT to 58 s, and PT to 52 s in AD-like plasma. The simultaneous prolongation of TT, APTT, and PT suggests that G-40 does not act exclusively on one upstream factor but likely modulates common pathway events and/or the thrombin-fibrinogen interface (table-2).

Table 2. Coagulation test results in control, AD-like, and G-40-treated plasma

Parameter	Control	AD-like model	AD-like model + G-40 (50 uM)
Thrombin time (TT), s	25	11	47
APTT, s	30	20	58
Prothrombin time (PT, TechPlastin), s	26	20	52

Values are presented exactly as reported in the dissertation-based experimental block.

Platelet calcium assays provided a mechanistic correlate for the antiaggregant effect. ADP stimulation increased intracellular Ca2+ release in platelets, whereas G-40 reduced this response in a concentration-dependent manner. Across the 10-100 uM range, G-40 inhibited ADP-evoked Ca2+ mobilization by 40-55%, with 50 uM representing the most effective concentration. In the AD-like condition, G-40 also lowered intracellular calcium relative to both baseline and ADP-stimulated values. The EGTA control further supported calcium-transport sensitivity in this assay system. Together, these data indicate that G-40 attenuates platelet activation at least partly through suppression of Ca2+-dependent signaling (table-3).

Table 3. Effects of G-40 polyphenol on ADP-induced platelet intracellular Ca2+ mobilization

Condition	Stimulus	G-40 concentration	Observed effect
Control platelets	ADP 5 uM	10-100 uM	ADP-evoked intracellular Ca^{2+} release inhibited by 40-55%; maximum effect at 50 uM
AD-like platelets	ADP 5 uM + EGTA control	50 uM	Intracellular Ca^{2+} level decreased relative to baseline and ADP-stimulated state; EGTA confirmed calcium-transport sensitivity

4. Discussion

The present results support a unified interpretation in which the AD-like condition promotes both platelet hyperreactivity and plasma hypercoagulability, while G-40 acts in the opposite direction on both compartments. The appearance of spontaneous aggregation and the increased responses to ADP, adrenaline, and collagen indicate that platelets in the AD model are pre-activated or primed. Mechanistically, such a phenotype is consistent with Abeta-driven platelet signaling, oxidative stress, enhanced autocrine agonist release, and disturbed calcium homeostasis [1-7,16-18]. Because ADP- and

collagen-driven pathways converge on phospholipase C activation, IP3 formation, intracellular calcium mobilization, and alphaIIbbeta3 activation, the calcium-lowering effect of G-40 provides a plausible downstream explanation for its antiaggregant action.

The coagulation results extend this interpretation from platelet function to plasma hemostasis. TT shortening in the AD-like condition indicates acceleration at the terminal thrombin-fibrinogen step, whereas concurrent APTT and PT shortening suggests a broader procoagulant shift. In AD-related vascular pathology, Abeta interactions with fibrinogen and coagulation factor XII, together with inflammatory and oxidative changes, have been linked to abnormal clot formation and impaired fibrinolysis [2-4]. Within this context, the ability of G-40 to prolong TT, APTT, and PT suggests that its action is not confined to one coagulation branch. The simplest mechanistic explanation is a multi-target anticoagulant profile involving common-pathway slowing (FXa -> thrombin -> fibrin) and/or interference with thrombin activity or fibrin polymerization. This interpretation remains inferential because direct enzymatic assays for thrombin or FXa were not performed.

An additional strength of the study is the convergence of three independent functional domains: aggregation, coagulation, and intracellular calcium. These domains point in the same biological direction and therefore reinforce each other. At the same time, the study has limitations. The exact molecular target of G-40 was not identified directly. No receptor-binding assays, thrombin amidolytic assays, FXa activity measurements, or flow-cytometric platelet activation markers were included. Moreover, the dissertation reported several model-development stages, whereas the present article focuses on the platelet/hemostasis block of the work. Therefore, the mechanistic model should be interpreted as evidence-based but still provisional.

5. Conclusions

The experimental AD-like condition was associated with spontaneous platelet aggregation, amplified agonist-induced platelet aggregation, enhanced intracellular Ca2+ mobilization, and shortening of TT, APTT, and PT, altogether indicating a prothrombotic peripheral phenotype. G-40 polyphenol at 50 uM partially inhibited platelet aggregation, suppressed ADP-induced intracellular calcium release, and markedly prolonged all coagulation times examined. These findings support the conclusion that G-40 exerts a multi-target antiaggregant and anticoagulant action under AD-like conditions, with the most consistent mechanistic explanation being attenuation of Ca2+-dependent platelet signaling together with modulation of common coagulation pathway events. G-40 may therefore represent a promising bioactive compound for correcting platelet hyperreactivity and hemostatic imbalance associated with AD.

Acknowledgment

This work was supported by the Applied Research Program of the Ministry of Higher Education, Science and Innovation of the Republic of Uzbekistan (project AL-27-4722022401 “Creation of a new drug with neuroprotective properties based on the raw materials of local plants Rhus typhina, Pinus sylvestris L., Hippophae rhamnoides L.”).

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